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ISOLATION AND CHARACTERISATION OF VASICINE FROM *IN VITRO* CULTURES OF *JUSTICIA ADHATODA* L.

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ABSTRACT

Justicia adhatoda L. which belongs to the family of Acanthaceae contains alkaloids like vasicine, vasicinone and deoxyvasicine. These alkaloids give the plant its expectorant activity, antispasmodic, antiseptic and antihelmintic properties. Hence, in the present investigation attempts were made to isolate and characterise alkaloids from callus and suspension cultures of *J. adhatoda*. Alkaloids were extracted with methanol, quantified and identified by colour reactions, Thin layer chromatograms (TLC), High performance liquid chromatogram (HPLC) and Fourier transform infrared spectroscopy (FT-IR) using vasicine as standard.

KEY WORDS : ***Justicia adhatoda*** **L., vasicine, expectorant, High performance liquid chromatogram, Fourier transform infrared spectroscopy.**

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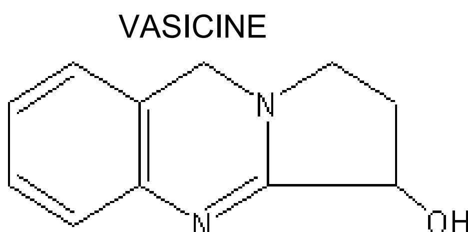
INTRODUCTION

Justicia adhatoda L. Syn. *Adhatoda vasica*, is one of the most important medicinal shrubs in South Asian traditional medicine. It is well known for its use in respiratory ailments. In traditional medicine system of India this plant is considered like mother to doctors. Hence the name Vaidyamata Singhee is given to plant in Sanskrit[1]. The plant leaf is valued for containing bronchodilator alkaloids, mainly vasicine, quinazoline, vasicinone, deoxyvasicine etc [2]. It is a valuable antispasmodic, antiseptic and antihelmintic plant. The roots and flowers also contained various alkaloids.

The main chemical constituents of *J. adhatoda* are vasicine, 2'-hydroxy-4-glucosyloxychalcone, vasicol, deoxyvasicinone (from leaves), vasicinone (from leaves, stem and roots), vasicinol (from stem and roots) etc. Vasicine is the major, as well as, the most important active principle of this medicinal plant**.** It is a quinazoline type alkaloid. It is an optically active molecule in its natural condition but, gets racemized when extracted.

J. adhatoda and its phytoconstituents including vasicine and its derivatives have . great potentials to develop safe medications for respiratory and reproductive medicine. Vasicine has antioxidant and anti-inflammatory activity[3,4]. Vasicine and vasicinone as the major alkaloids in the extract of this plant were suggested to play a key role in the radioprotective/genoprotective activity of this plant[5]. It was observed that vasicine potentiated the action of oxytocin in isolated rat mammary strip preparation and showed smooth muscle stimulant activity and is thus used for bronchodilation and abortion[6]. The uterotonic effect of vasicine was similar to that of methergen and pitocin, two known oxytocics[7].

Vasicine can be used for controlling the capillary haemorrhages and correction of drug induced bone marrow depression[7]. This alkaloid and its semi-synthetic derivatives, benzylamines, bromhexine and ambroxol, showed anticestodal activity[8]**,** antileishmanial activity **[9]**, anti-helminthic activity **[10]**, anti-bacterial activity and mucus liquefying or expectorant activity[11]



Molecular Formula = $C_{11} H_{12} N_2 O$

Formula Weight = 188.226

Ambroxol hydrochloride is a metabolic product of bromhexine. It is a secretolytic agent and found to inhibit IgE-dependent mediator secretion from human mast cells and basophils, which are the main effector cells of allergic inflammation[12]. There are several studies describing various methods for extraction of vasicine from *J. adhatoda* and a comparison of these methods was done [13].

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MATERIALS AND METHODS

(i) Plant material:

Healthy, disease free, tender twigs (4-5 cm) and roots of mature *J. adhatoda* were collected from Central Kerala, India. The specimen was authenticated by Dr. V T Antony, Head, Department of Botany, St. Berchmans College, Changanacherry and a voucher specimen was deposited at Regional Herbarium Kerala with field no. RHK 6301 and accession no. 7564.

(ii) In vitro culture:

Plant materials were washed under running tap water followed by washing with few drops of Tween 20 (E. Merck (India) Limited) for 15-30 minutes. Then disinfected by immersing in 0.1% mercuric chloride (E. Merck (India) Limited) solution for 3-5minutes and after several rinses with sterile distilled water inoculated onto the MS (Murashige and Skoog) medium supplemented with Indole 3- acetic acid (IAA) (6mgL^{-1}) and Kinetin (Kn) (6mgL^{-1}) for leaf, Indole 3- butyric acid (IBA) (3mgL^{-1}) and 6-Benzylaminopurine (BA) (3mgL^{-1})for axillary bud and IBA (3mgL^{-1}) and BA (6mgL^{-1}) for root tip explants. Cultures were maintained at 25±2°C under 16/8hr photoperiod of 2000lux light intensity with 55%-60% humidity.

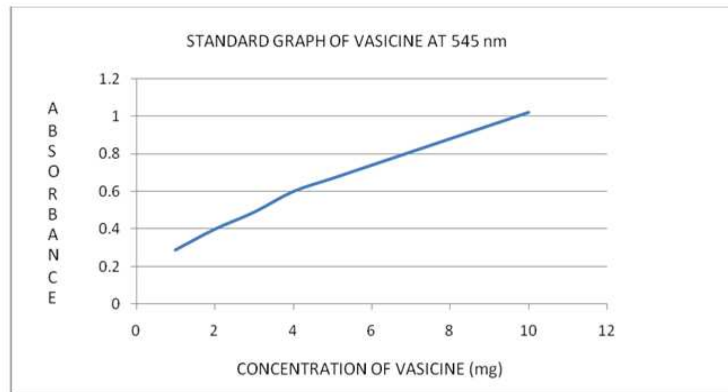
(iii) Extraction and characterisation of secondary metabolite:

Friable calluses were transferred to 50ml of MS medium and incubated on a rotary shaker at 120 rpm. For extraction of secondary metabolite, methanol extract of callus (500mg in 10 ml) (VAS I) and supernatant of suspension culture (1:1) (v/v) (VAS II) were used. Thin layer chromatograms (TLC)[14] were developed with Ethyl acetate: Methanol: Water (100:13.5: 10) on a silica gel (Merck silica gel 60) plate. The colour of the spots was noted with Dragendorff's reagent and the Rf values were calculated. The spots were separately scrapped off, and the individual fraction isolated was identified and quantified using spectrophotometric analysis [13], High performance liquid chromatogram (HPLC) [14]and Fourier transform infrared spectroscopy (FT-IR) [14] using vasicine (SPIC India Ltd, Chennai) as standard.

(iv) Quantification of total alkaloids:

Quantification of total alkaloids was done by spectrophotometric method with tropaeolin 'OO' (SIGMA ALDRICH). Coloured complex developed was measured at 545 nm against blank. The amount of total alkaloids in the samples was calculated using standard curve of vasicine (Fig.1). The content of the total alkaloids was expressed as vasicine [13].

Figure 1
Standard graph of vasicine



RESULT AND DISCUSSION

Friable soft callus was obtained from leaf, axillary bud and root tip explants and it was inoculated into the suspension culture. It showed cell separation and multiplication of callus. In the present investigation an alkaloid was found in callus as well as in the suspension cultures. A steady increase in production of this alkaloid occurred from the day of inoculation to the 15th day in the case of suspension culture and decreased after that (Fig.2). Upon quantification of alkaloid production during different time periods, it was observed that initially maximum vasicine content was found in root tip callus suspension but maximum vasicine production observed on 15th day of suspension culture was not different in the case of suspension cultures of root tip, axillary bud and leaf calli

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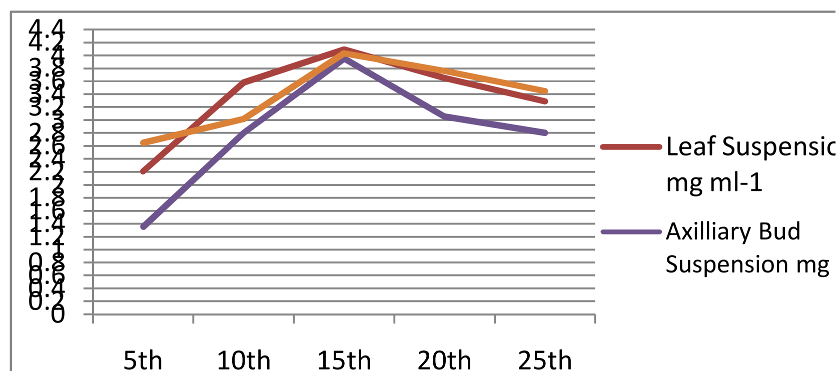
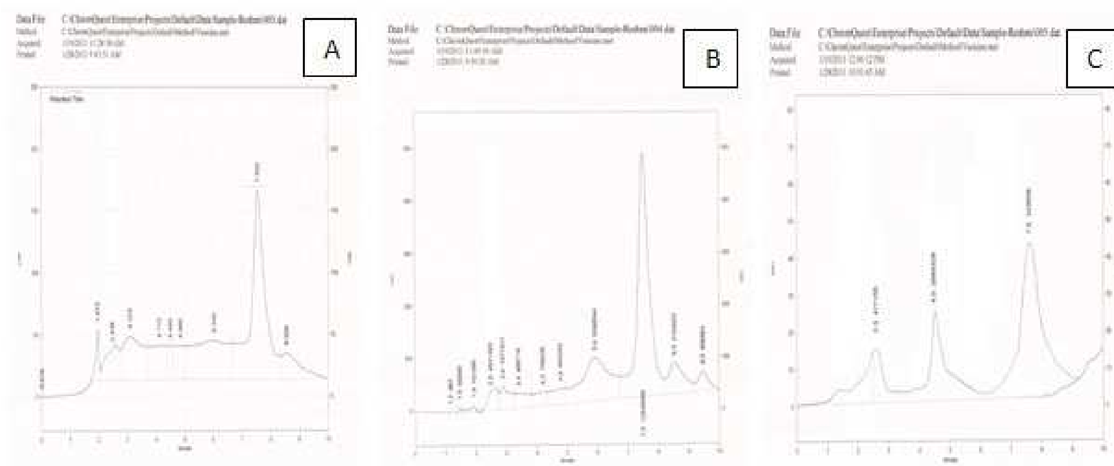
Figure 2
The concentration of vasicine in callus and suspension cultures of J. adhatoda in different growth periods.



The secondary metabolite present in *J. adhatoda* was first confirmed as alkaloid by preparative TLC. In this study the detection of alkaloid in the samples in TLC plate was done under UV light of 245 and 365 nm and identified based on their colour and Rf values. Alkaloid develops orange colour with Dragendorff's reagent[15]. Rf value obtained is 0.4, which corresponds to that of vasicine [13]. These spots were scrapped off, dissolved in methanol (E. Merck (India) Limited) and used for HPLC and FT-IR analysis using vasicine as standard.

HPLC chromatogram of the sample showed retention time similar to standard vasicine

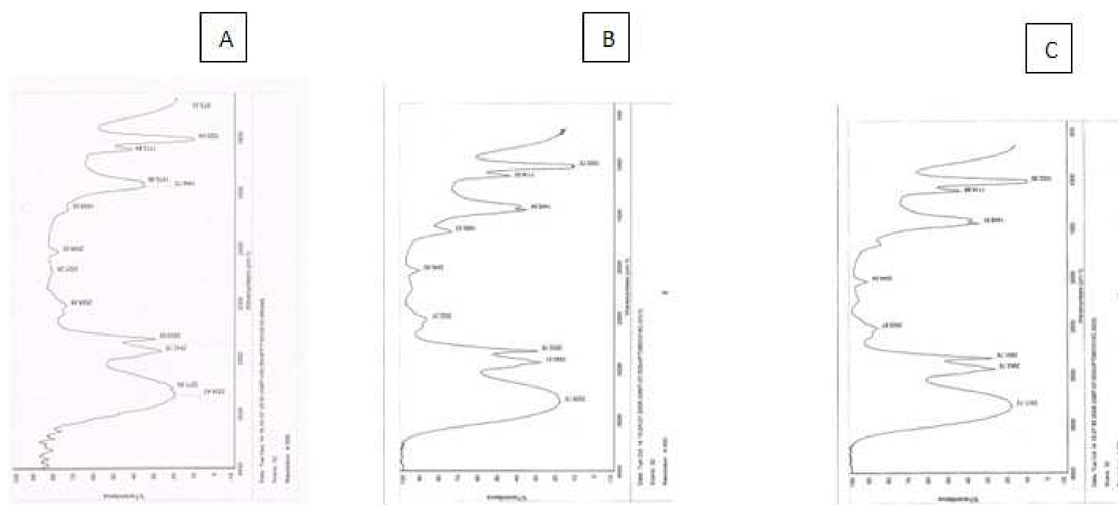
.Figure 3
HPLC chromatogram



A. HPLC Chromatogram of Standard, B. HPLC Chromatogram of VAS II, C. HPLC Chromatogram of VAS I

For the conformation of spectral characteristics, FT-IR spectroscopy of the extracts was done using vasicine as standard. In the FT-IR spectrum of the extracts examined, a characteristic broad band of range 3000-3600 cm^{-1} was observed which was indicative of aromatic hydrogen and aromatic hydroxyl groups. The band of 1660 cm^{-1} might be indicating the presence of saturated cyclic C-O or keto-enol conformation. Also bands at wave numbers 1020 cm^{-1} might be indicative of C-N, 1114 cm^{-1} might be of C-H and 1448 cm^{-1} might be of aromatic hydrogen present in the suspected molecule. FT-IR spectra of the extracts were comparable with that of standard.

Figure 4
FT-IR spectra



A. FT-IR Spectrum of vasicine standard, B. FT-IR Spectrum of VAS I, C. FT-IR Spectrum of VAS II

QUANTIFICATION OF VASICINE

Vasicine content was found to be 5.15mg ml^{-1} and 4.09mg ml^{-1} in callus and suspension cultures of leaf respectively which were on par with field grown plants [13]. 5.08mg ml^{-1} and 3.95mg ml^{-1} of vasicine and 5.05mg ml^{-1} and 4.03mg ml^{-1} of vasicine were found in callus and suspension cultures of axillary bud and root tip respectively. All the observations strongly support the presence of vasicine in the extract of callus and suspension cultures of various explants. The results obtained in the present work were promising and suggest a viable, alternative, simple and rapid methodology for isolation of vasicine from *J. adhatoda* callus cultures.

CONCLUSION

In the present study an efficient protocol has been developed for the production of vasicine as a secondary metabolite through callus and suspension culture of *J. adhatoda* L. Separation, purification and characterization of vasicine have also been done by TLC, HPLC and FT-IR spectroscopy.

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